EDITORIAL

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Return of the malingering mutants

M Greaves*,[1]

[1]Center for Evolution and Cancer, The Institute of Cancer Research, Brookes Lawley Building, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK

Of all the hallmark biological features of cancer, drug resistance stands out as the harbinger of bad news for patients and oncologists alike. Cancer cells can employ several adaptive mechanisms for evading chemotherapeutic assault (Redmond *et al*, 2008) (Table 1). Prominent among these is mutation of the gene(s) encoding the drug targets. Unambiguous and consistent evidence for this route to escape has been provided in the recent era of therapy with small-molecule tyrosine kinase inhibitors (TKIs) (Gorre *et al*, 2001; Kosaka *et al*, 2006). Despite the extraordinary success of imatinib for the treatment of chronic myeloid leukaemia (CML), many patients, particularly with more advanced disease, relapse with imatinib-resistant *ABL1* mutations (Gorre *et al*, 2001; Branford *et al*, 2002; Shah *et al*, 2002). More than 50 distinct mutations have been described, all impairing drug binding to the *ABL1* kinase domain active site (Schindler *et al*, 2000; Shah *et al*, 2002). Although such mutations have the appearance of being adaptively acquired in response to therapy, this is not the underlying mechanism. As in any Darwinian evolutionary system of natural selection, for example, speciation in ecosystems, antibiotic resistance in bacteria (Lambert *et al*, 2011), mutations accrue in a stochastic or random manner with respect to the functions encoded by the mutant gene. A vast majority of them are destined to remain neutral in impact and will be present in usually undetectable, small subclones. The probability of a specific drug-resistant mutation arising will be a function of the intrinsic mutability of that locus and the number of proliferative 'at-risk' cycles in self-renewing cancer stem cells – the necessary repository of selectable mutations (Greaves, 2013). In addition, and critically, if the cancer has acquired genetic instability, this will greatly accelerate the rate of mutation accrual. This probability of an *ABL1* kinase mutation being present at diagnosis of CML has been calculated, albeit making assumptions about the above parameters, the numbers for which that will have wide confidence limits. These analyses suggested that ~10–100% of patients with CML will have *ABL1* kinase mutations on board before instigation of TKI therapy, depending upon stage of disease (Michor *et al*, 2005). The *BCR–ABL1* kinase activity has been associated with ROS (Nieborowska-Skorska *et al*, 2012) and increased genetic instability or mutation frequency (Salloukh and Laneuville, 2000), and this may accelerate the rate of acquisition of *ABL1* kinase mutations as well as other 'driver' or oncogene mutations that promote the acute or blast crisis phase of disease.

The emergence of TKI-resistant mutants, in relapse, is then the consequence of the positive selective pressure provided by the specific drugs: the rare and covert mutant clone now finds itself as a beneficiary of therapy with an enormous competitive advantage in terms of ecosystem space and resources, whereas its clonal relatives are decimated. Evidence for this sequence of events comes from the finding of low-level, drug-resistant mutations in both CML (Roche-Lestienne *et al*, 2002) and *BCR–ABL1*-positive ALL (Pfeifer *et al*, 2007), T-ALL (Meyer *et al*, 2013) or colorectal cancer (Diaz *et al*, 2012) before the exposure to the drugs that subsequently elicited their clonal dominance.

This much follows simple and predictable evolutionary paths. But what happens to such emergent drug-resistant clones if the therapy is then switched to a drug to which they are sensitive? The expectation is that, following de-selection, they would dramatically decline to very low levels or become extinct – depending upon the efficacy of the new drug or drug regime.

In this issue, Parker *et al* (2013) provide some intriguing insight into the oscillating fate of *ABL1* kinase mutations. Five patients with imatinib-resistant CML were serially followed throughout switches in therapy that involved other *ABL1* kinase inhibitors (dasatinib, nilotinib) or bone marrow transplantation. Although the details vary with the different patients, in principle the data illustrate that the imatinib-resistant mutant clone that predominates in initial recurrence of disease declines to undetectable levels when de-selected but can reappear when the therapy, for one reason or another, is changed again (Figure 1). The authors consider the probability that the recurrent mutant is a second, independent version of the same initial mutation but plausibly argue that this is unlikely. The result begs two questions. First, is it surprising that the mutant clone lingers on in a covert manner with its latent malignancy de-selected? The answer must be no. The new *AML1* kinase inhibitor or alternative therapy may fail to eliminate all CML cells irrespective of their *ABL1* kinase mutant status; plus quiescent CML stem cells, mutant or not, appear to be remarkably resistant to *ABL1* kinase inhibition (Jiang *et al*, 2007). Hanfstein *et al* (2011) previously reported oscillating selection, de-selection (but regularly detectable) and re-selection in patients in whom TKIs were alternated with other chemotherapies. What is more surprising is that the de-selected clone should return to dominance in the absence of the specific drug that elicited its emergence in the

*Correspondence: Professor M Greaves; E-mail: mel.greaves@icr.ac.uk

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Table 1. Means of therapeutic escape

1.	Genetic instability	Mutation in target (or in drug uptake/efflux pathway)[a]
2.	Target redundancy	Signal bypass of target dependence (or addiction)[b]
3.	Stem cell plasticity	Quiescent cancer stem cells are generally chemoresistant (Saito *et al*, 2010)
4.	Subclonal diversity	Cancer subclones and their constituent stem cells are genetically diverse and some may lack related drug target (Anderson *et al*, 2011; Greaves and Maley, 2012).[c]

[a]By amplification of target or mutational loss of drug-binding site.
[b]As a result of target redundancy in signalling network (Sharma *et al*, 2010; Workman and Clarke, 2011; Prahallad *et al*, 2012; Wilson *et al*, 2012) or selection for subclone with another mutation that facilitates bypass of target (Engelman *et al*, 2007).
[c]This escape route applies particularly to highly targeted therapies aimed at mutant proteins or specifically dysregulated pathway proteins. However, this escape mechanism would not apply if the therapeutic target was ubiquitously expressed in the cancer, for example, as an addictive founder mutation – as in *BCR–ABL1* in CML.

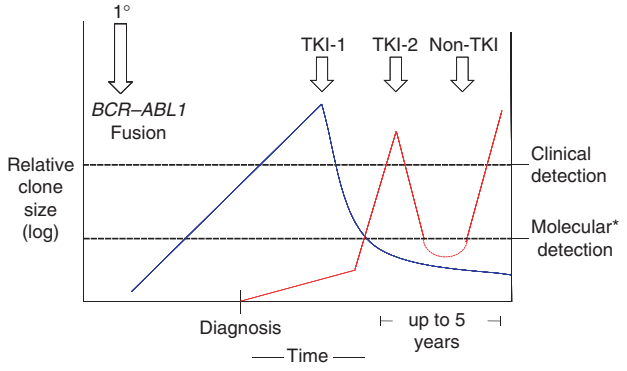

Figure 1. Patterns of sequential clonal dominance in CML treated with kinase inhibitors. Shifting patterns of clonal dominance seen in several patients reported by Parker *et al* (2013) are summarized. Tyrosine kinase inhibitor 1 (TKI-1, for example, imatinib) and tyrosine kinase inhibitor 2 (TKI-2, for example, desatinib). *By Sanger sequencing: 10–20% sensitivity or by mass spectrometry: 0.2% sensitivity.

first place. One possible explanation for this is that the mutant clone may have been less sensitive to the second-line TKI (O'Hare *et al*, 2005) and hence at a clonal level retained competitive advantage. Another is that some *ABL1* kinase mutants ironically have more potent oncogenic activity (Shah *et al*, 2007) and this gives them the edge. Whatever the biological explanation, a clear practical inference from the observation of Parker *et al* (2013) is that sensitive molecular screening for residual, specific drug-resistant mutations would be informative and help dictate choice of therapy – for CML and any cancer where a limited range of resistance genotypes can emerge in response to highly targeted therapy. This would be relatively straightforward for blood-borne leukaemia cells but more demanding for solid tumours where biopsies are likely to be a biased sample of a cancer with topographical segregation of subclones (Gerlinger *et al*, 2012; Greaves and Maley, 2012). However, there are potential solutions to this dilemma. If a limited range of mutations are normally positively selected by therapy, then these might be detectable, before therapy, by sensitive screens of DNA fragments in plasma (Murtaza *et al*, 2013). Alternative generic measures of clonal diversity may provide a practical surrogate for the probability than any drug-resistant mutants exist (Mroz *et al*, 2013).

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